which represents the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP) as a pharmacologically active antitumor agent. The peptide is about 50% as active as the protein per se but only about one-tenth of the weight of the peptide is equivalent in activity of the protein (ANUP) on a molar basis (ca 10^{-9}M).

SUMMARY OF THE INVENTION

The present invention describes the pharmacologically anti-tumor activity of the 16 amino acid peptide which represents the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP).

The 16 amino acid peptide is approximately one-half as active as the protein on a molar basis utilizing the human breast tumor cell line (MDA 231). However, only about one-tenth of the weight of the peptide is required when compared to the amount of protein for equivalent activity against the human breast tumor cell line. Both the protein and the peptide exert their action by killing the tumor cells (apoptosis) since electron microscopy studies showed complete degradation of the cells (Struve et al. Cancer Res. Therapy and Control (1990) 1: pp 225-230.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The 16 Amino Acid Peptide

~~The synthetic hexadeca peptide (16 L-amino acids) has the following sequence:~~ Of

1.	Pyroglu		9.	Glu	E
2.	Leu	L	10.	Pro	P
3.	Lys	K	11.	Met	M
4.	Cys	S	12.	Thr	T
5.	Tyr	Y	13.	Ser	S
6.	Thr	T	14.	Ala	A
7.	Cys	C	15.	Ala	A
8.	Lys	K	16	Cys	C

The peptide was synthesized by Research Genetics Inc., Huntsville, AL 35801; the peptide was pure as shown by HPLC (high performance liquid chromatography) and the molecular weight was checked by mass spectrometry (MS).

The pharmacological anti-tumor activity of the 16 amino acid peptide (P_{16})

The antitumor activity of the peptide (P_{16}) was assayed against the human breast tumor cell line (MDA 231) and its activity was compared to the in vitro antitumor effect of the "pure" protein (ANUP).

The assay for the pharmacological antitumor activities were performed as follows utilizing 96 well plates --

20,300 - 30,000 human breast tumor cells in L-15 medium (200 ul) containing 2.5% fetal calf serum and 100 ug gestamycin per ml (complete medium) were incubated at 37° in air for 120 hours; after this incubation period 50 ul of serially diluted P_{16} and ANUP were added to each well. The serial dilutions were prepared as follows: 2 mg each (the P_{16} and ANUP) were dissolved in 2 ml of complete medium containing 0.5% sodium dodecyl sulfate (SDS). The solutions were diluted in complete medium containing 0.05% SDS to a concentration of 350 ug per ml.

Dilution plates were prepared as follows:

100 ul of complete medium were added to each well and 50 ul of diluted P_{16} and ANUP were added to each well in row A thus 1.3 dilution was accomplished; 50 ul were serially diluted in the 100 ul of medium in rows B through H. Thus the range of concentrations were from 6 ug to 2 mg when 50 ul each dilution series were added to 200 ul of the complete medium containing the MDA cells. The plates were incubated for an additional 96-120 hours. The medium was poured off and after a 90-minute incubation with 50 ul neutral red dye (0.5 ml neutral red (0.25% in 25% ethanol (0.6 ml) diluted 5.5 saline - 0.16 mm HCl) the cells were washed twice with PBS (phosphate buffer saline) at room temperature. The concentration of living cells (since only living cells absorb the dye) was determined after adding 100 ul lysing buffer (50% ethanol in 0.05 m NaH_2 PO_4) the concentration of neutral red released in each well was determined using a Dynetech plate reader set at 550 mm. A unit of activity was defined as the concentration of ANUP and P_{16} for 50% killing.

Under these assay conditions the 50% end points were as follows:

ANUP 0.1 ug/well = 1.25×10^{-8}M

P_{16} 0.0 ug/well = 2.2×10^{-8}M

Thus P_{16} is about 50% as active as ANUP on a molar basis; whereas on a weight basis only one tenth of the peptide weight is equal in activity 10 times the weight of the protein (ANUP).